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Development of *Toxoplasma gondii* vaccine

A global challenge

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Toxoplasmosis is caused by the protozoan parasite *T. gondii*. Humans and other warm-blooded animals are its hosts. The infection has a worldwide distribution; one-third of the world's population has been exposed to this parasite. There are three primary ways of transmission: ingesting uncooked meat containing tissue cysts, ingesting food and water contaminated with oocysts from infected cat feces and congenitally. Those particularly at risk of developing clinical illness include pregnant women, given that the parasite can pose a serious threat to the unborn child if the mother becomes infected while pregnant, and immunosuppressed individuals such as tissue transplant subjects, AIDS subjects, those with certain types of cancer and those undergoing certain forms of cancer therapy. Maternal infections early in pregnancy are less likely to be transmitted to the fetus than infections later in pregnancy, but early fetal infections are more likely to be severe than later infections. In the absence of an effective human vaccine, prevention of zoonotic transmission might be the best way to approach the problem of toxoplasmosis and must be done by limiting exposure to oocysts or tissue cysts. Vaccine development to prevent feline oocyst shedding is ongoing, mostly with live vaccines. The S48 strain Toxovax is a live vaccine originally developed for use in sheep, but when used in cats inhibits sexual development of *T. gondii*. This vaccine is used in sheep to reduce tissue cyst development. The T-263 strain of *T. gondii* is a live mutant strain designed to reduce or prevent oocyst shedding by cats by developing only partial infection in the feline intestinal tract.

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Development of *Toxoplasma Gondii* Vaccine: A Global Challenge

Toxoplasmosis is caused by the protozoan parasite *T. gondii*. Humans and other warm-blooded animals are its hosts.[1] Approximately one-third of all humans have been exposed to this parasite. Although usually asymptomatic in immunocompetent adults, it can cause severe disease manifestations and even death in immunocompromised subjects. If acquired during pregnancy, it can cause various congenital anomalies in the child.[2,3] In India, seroprevalence of this infection is unknown. However, using various diagnostic tests, prevalence has been reported to be as low as 5% and as high as 80% in adults. There is lack of awareness and knowledge about this zoonotic infection. Several commercial organizations are further complicating the situation by promoting their products without proper background knowledge and baseline data from India. This commentary brings out important and relevant aspects of toxoplasmosis in India.[4]

There are three primary modes of transmission: ingesting uncooked meat containing tissue cysts, ingesting food and water contaminated with oocysts from infected cat feces (as encountered in gardens and children's sand pits) and congenitally. Toxoplasmosis is recognized to be a water-borne zoonosis. This method of transmission occurs where water treatment is ineffective or non-existent and there is a

sizable local felid population that contaminates surface water with oocysts. Linked to this is an appreciation that sea mammals are becoming infected by waters from contaminated land and from untreated urban sewage. Transplacental infection occurs when an uninfected mother acquires infection during pregnancy. First there is parasitemia in the mother, then invasion of placenta and finally spreads to fetal tissues. Overall, < 0.1% of the general population becomes infected congenitally. The parasite also can be transmitted by transplantation of organs and blood transfusion. Poor hygiene observed in India during handling of meat from slaughterhouse to kitchen can be a source of infection.[5,6] Until recently prevalence of *T. gondii* in the general Indian population was considered to be low compared with Western countries.[7,8]

T. gondii readily infects humans, but clinical illness is relatively uncommon. Those particularly at risk of developing clinical illness include pregnant women, since the parasite can pose a serious threat to the unborn child if the mother becomes infected while pregnant, and individuals who are immunosuppressed, such as subject with tissue transplants, AIDS, certain types of cancer and undergoing certain forms of cancer therapy. These individuals are at risk of developing acute lethal infection if left untreated. The very young and very old may also be more susceptible. Some people with no apparent immune deficiency may develop an illness characterized by general malaise, fever and lymphadenopathy.[9]

It needs to be re-emphasized that women who are seropositive before conception have the least risk to their babies, if at all. Women infected with before conception, with rare exceptions, do not transmit the infection to the fetus. Women infected after conception (i.e., during pregnancy) can transmit the infection across the placenta. Maternal infections early in pregnancy are less likely to be transmitted to the fetus than later in pregnancy, but early fetal infections are more likely than later infections to be severe.

An estimated one-half of untreated maternal infections are transmitted to the fetus.[10] Of these fetal infections, 60% are subclinical, 9% are fatal and 30% have severe damage such as hydrocephalus, intracerebral calcification, retinochoroiditis (Classical triad) and mental retardation. If a woman's fetal loss is due to *T. gondii* infection, her subsequent pregnancies are safe as far as this infection is concerned, unless she becomes immunocompromised subsequently. However, there have been occasional reports of congenital toxoplasmosis transmitted by an immunocompetent woman infected before conception.[11]

Prevention of Infection in Humans

In the absence of an effective vaccine for humans, prevention of zoonotic transmission might be the best way to approach the problem of toxoplasmosis, and must be done by limiting exposure to oocysts or tissue cysts. Recommendations for accomplishing this include practicing good hygiene (e.g., washing hands after soil contact, washing raw fruits and vegetables), freezing meat at -12°C for 24 h[12] and/or cooking meat until an internal temperature of 66°C is reached, and not drinking untreated water. Cats should be kept indoors, fed commercially prepared diets and litter boxes cleaned daily, given that it takes at least one day for organisms to sporulate and become infectious after shedding. Recommendations speciðcally for pregnant women include wearing gloves when gardening or contacting soil or sand, followed by thorough hand washing. Pregnant women also should avoid changing cat litter. Owners should keep dogs away from litter boxes to prevent oocyst ingestion.[13,14] Health education for women of childbearing age should include information about meat, soil and cat feces for preventing infection. Immunosuppressed persons, including those with HIV infection, should be educated about preventing infection. Veterinarians should educate their canine-owning clients of the importance in vaccinating against distemper, since most cases of canine toxoplasmosis are seen in those not vaccinated against this immunosuppressive virus.[15]

Vaccines

Vaccine development to prevent feline oocyst shedding is ongoing, mostly with live vaccines. Disadvantages include limited shelf life and risk of infection to humans handling the vaccines. The S48 strain Toxovax is a live vaccine originally developed for use in sheep, but when used in cats inhibits sexual development of *T. gondii*. This vaccine is used in sheep to reduce tissue cyst development. The T-263 strain of *T. gondii* is a live mutant designed to reduce or prevent oocyst shedding by cats by developing only partially in the intestinal tract. Field trials with this vaccine were conducted on US pig farms. Cats were trapped, vaccinated and released. After vaccination of resident farm cats, *T. gondii* seroprevalence decreased in farmed pigs, suggesting less environmental contamination with oocysts, and thus less infection risk for the pigs. There are reports of vaccination of cats using *T. gondii* strains modiðed by irradiation, chemical treatment, selected recombinant antigens and new delivery systems, including a feline herpes-virus type 1 vehicle for delivery. All of these vaccines confer a level of immunity to cats, but a need exists for a killed or recombinant vaccine that could serve as a model for a human vaccine.[16,17]

Toxoplasmosis in an immunocompetent host induces lifelong protective immunity to reinfection. Some protective antigens are candidates for vaccine development. Different antigens were entrapped within liposomes and evaluated for their ability to protect Swiss mice against infection: soluble tachyzoite antigen (L/TAg), tissue cyst (L/CAg), tachyzoites plus tissue cyst (L/TCAg) or purified tachyzoite antigen (L/pTAg). The protein used in L/pTAg was purified from tachyzoites using a stage-specific monoclonal antibody that reacted with a 32kD protein. To compare the adjuvant action of liposomes and of Freund's Complete Adjuvant (FCA), another group of mice was immunized with soluble tachyzoite antigen (STAg) emulsified in FCA (FCA/TAg). Control groups were inoculated with STAg alone, phosphate-buffered saline (PBS), FCA with PBS (FCA/PBS) and empty liposomes (L/PBS). Mice were inoculated subcutaneously with these antigens at six, four and two weeks before oral challenge with 80 tissue cysts of the P strain of *T. gondii*. All mice immunized with or without adjuvant showed a humoral response as measured by ELISA. However, no

correlation was found between antibody titer and protection against challenge. All mice immunized with L/pTAg or L/TCAg survived, whereas 80–90% of mice from groups that received PBS, FCA/PBS or L/PBS died. All mice immunized with antigen in liposomes (L/TAg, L/CAg, L/TCAg and L/pTAg) showed low numbers of intracerebral cysts. A *T. gondii* vaccine may make employ both the dense granule protein GRA2 and surface antigen 1 (SAg1) from the organism.[18]

The use of DNA vaccines and vaccines with live bacterial components has ethical issues for humans, but such a vaccine also has a vast market. Another vaccination strategy is to immunize hosts before pregnancy and generate a protective response that will last during pregnancy, thus preventing the fetus from harm. Beauvillain et al.[19] generated a vaccine using a vesicle secreted by *T. gondii* that is cell-free and contains antigenic properties. They saw a protective response against challenge with *T. gondii*, indicating that this is a possible human vaccine. It is unclear how this secreted vesicle elicits a specific immune response, so more work needs to be done to determine mechanism of action, but this is a great stride toward a human vaccine. The benefits of prophylactic vaccination could be: (1) Prevention of human infection or at least of clinical disease; (2) Prevention of infection in animals raised for human consumption, thereby preventing transmission; (3) Immunization of cats to disrupt the zoonotic cycle and prevent contamination of the environment by oocysts. In principle, an effective recombinant vaccine against both sexual and asexual stages of the parasite should be able to address all three targets, but this is hampered by stage-specific expression of *T. gondii* proteins.[19]

Minimum guidelines should be followed for vaccine development: (1) Vaccine constructs aiming at protection against cyst-forming *T. gondii* in animal models should have brain cyst load as a main endpoint, not survival; (2) Infection with cysts simulating oral infection can be done using brain emulsion, but this needs to be administered orally; (3) Challenge should be performed in parallel, using at least two *T. gondii* isolates of different lineages. Protection against the less pathogenic genotype II is not enough to demonstrate protective immunity against other genotypes; (4) Immunization should be performed in a number of inbred mouse strains, preferably BALB/c and C57BL/6 or C3H/HeN; (5) Vaccine efficacy also should be tested in outbred mice, since this more closely reflect the genetic situation of animals and humans.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

1. Dubey JP. Toxoplasmosis - a waterborne zoonosis. Vet Parasitol 2004; 126:57-72; PMID:15567579; http://dx.doi.org/10.1016/j.vetpar.2004.09.005.
2. Lamb GA, Feldman HA. Risk in acquiring toxoplasma antibodies. A study of 37 "normal" families. JAMA 1968; 206:1305-6; PMID:5695801; http://dx.doi.org/10.1001/jama.1968.03150060079023.
3. Evengård B, Lilja G, Capraru T, Malm G, Kussofsky E, Oman H, et al. A retrospective study of seroconversion against Toxoplasma gondii during 3,000 pregnancies in Stockholm. Scand J Infect Dis 1999; 31:127-9; PMID:10447319; http://dx.doi.org/10.1080/003655499750006146.
4. Singh S. Mother-to-child transmission and diagnosis of Toxoplasma gondii infection during pregnancy. Indian J Med Microbiol 2003; 21:69-76; PMID:17642985.
5. Singh S, Singh N, Maniar JK. AIDS Associated Toxoplasmosis in India and its correlation with serum tumor necrosis factor-alpha. J Parasit Dis 1996; 20:49-52.
6. Singh S, Singh N. Seroepidemiology of toxoplasmosis in sheep and goats of Rajasthan state and their butchers. In: Somvanshi R & Lokeshwar RR. (Eds.). Current advances in Vaterinary Sciences and Animals Production in India. (International Book Distributing Co., Lucknow) 1994:204-12.
7. Bowerman RJ. Seroprevalence of Toxoplasma gondii in rural India: a preliminary study. Trans R Soc Trop Med Hyg 1991; 85:622; PMID:1780991; http://dx.doi.org/10.1016/0035-9203(91)90369-A.
8. Mittal V, Bhatia R, Singh VK, Sehgal S. Prevalence of toxoplasmosis in Indian women of child bearing age. Indian J Pathol Microbiol 1995; 38:143-5; PMID:8919098.
9. Bowie WR, King AS, Werker DH, Isaac-Renton JL, Bell A, Eng SB, et al.; The BC Toxoplasma Investigation Team. Outbreak of toxoplasmosis associated with municipal drinking water. Lancet 1997; 350:173-7; PMID:9250185; http://dx.doi.org/10.1016/S0140-6736(96)11105-3.
10. Holliman RE. Congenital toxoplasmosis: prevention, screening and treatment. J Hosp Infect 1995; 30(Suppl):179-90; PMID:7560949; http://dx.doi.org/10.1016/0195-6701(95)90018-7.
11. Villena I, Chemla C, Quereux C, Dupouy D, Leroux B, Foudrinier F, et al.; Reims Toxoplasmosis Group. Prenatal diagnosis of congenital toxoplasmosis transmitted by an immunocompetent woman infected before conception. Prenat Diagn 1998; 18:1079-81; PMID:9826901; http://dx.doi.org/10.1002/(SICI)1097-0223(1998100)18:10<1079::AID-PD391>3.0.CO;2-E.
12. Jones JL, Kruszon-Moran D, Sanders-Lewis K, Wilson M. Toxoplasma gondii infection in the United States, 1999 2004, decline from the prior decade. Am J Trop Med Hyg 2007; 77:405-10; PMID:17827351.
13. Mitchell SM, Zajac AM, Kennedy T, Davis W, Dubey JP, Lindsay DS. Prevention of recrudescent toxoplasmic encephalitis using ponazuril in an immunodeficient mouse model. J Eukaryot Microbiol 2006; 53(Suppl 1):S164-5; PMID:17169046; http://dx.doi.org/10.1111/j.1550-7408.2006.00217.x.
14. Lopez A, Dietz VJ, Wilson M, Navin TR, Jones JL. Preventing congenital toxoplasmosis. MMWR Recomm Rep 2000; 49(RR-2):59-68; PMID:15580732.
15. Lindsay DS, Dubey JP, Butler JM, Blagburn BL. Mechanical transmission of Toxoplasma gondii oocysts by dogs. Vet Parasitol 1997; 73:27-33; PMID:9477489; http://dx.doi.org/10.1016/S0304-4017(97)00048-4.
16. Centers for Disease Control and Prevention (CDC). Guidelines for Prevention and Treatment of Opportunistic Infections in HIV Infected Adults and Adolescents; Recommendations from CDC, the National Institutes of Health, and the HIV Medicine Association of the Infectious Diseases Society of America. MMWR 2009; 58 [No. RR-4]: 200–2.
17. Innes EA, Bartley PM, Maley S, Katzer F, Buxton D. Veterinary vaccines against Toxoplasma gondii. Mem Inst Oswaldo Cruz 2009; 104:246-51; PMID:19430650; http://dx.doi.org/10.1590/S0074-02762009000200018.
18. Elsaid MMA, Vitor RWA, Frézard FJG, Martins MS. Protection against toxoplasmosis in mice immunized with different antigens of Toxoplasma gondii incorporated into liposomes. Mem Inst Oswaldo Cruz 1999; 94:485-90; PMID:10446006; http://dx.doi.org/10.1590/S0074-02761999000400010.
19. Beauvillain C, Juste MO, Dion S, Pierre J, Dimier-Poisson I. Exosomes are an effective vaccine against congenital toxoplasmosis in mice. Vaccine 2009; 27:1750-7; PMID:19186199; http://dx.doi.org/10.1016/j.vaccine.2009.01.022.